Family Medicine and Community Health
ORIGINAL RESEARCH

Massage treatment of chronic persistent pediatric asthma

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Abstract

Objective: We observed the clinical effect of massage in treating chronic persistent pediatric asthma and the changes in expression of TLR1, TLR2, and TLR4 on macrophages.

Methods: One hundred sixty pediatric patients with persistent chronic asthma were collected, and divided into treatment and control groups of 80 each in strict accordance with the principle of random allocation. The patients in the control group received general nebulizer therapy, and the patients in the treatment group received infantile massage. Both groups of patients were observed for 3 months with respect to the following indicators: the frequency of asthmatic attacks; the frequency of respiratory tract infections (RTIs); C-ACT rating; and the PEF% of peripheral blood (PB) before and after treatment to detect macrophage TLR1, TLR2, and TLR4.

Results: After treatment, the frequency of asthmatic attacks, frequency of RTIs, C-ACT rating, and the PEF% and macrophage TLR1, TLR2, and TLR4 expression of fluorescence intensity in the treatment group showed a statistically significant increase when compared with the control group ($^{\star}P<0.05$).

Conclusion: Pediatric massage can improve the clinical symptoms of pediatric asthma by up-regulating the expression of macrophage TLR1, TLR2, and TLR4.

Keywords: Asthma, Pediatric massage, TLRs

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Introduction

Bronchial asthma is a common disease among children with repeated attacks due to various factors. TCM techniques, especially pediatric massage, play a significant role in the prevention and treatment of pediatric asthma [1–3]. As a result of long-term massage of children with asthma, the expression of macrophage TLR1, TLR2, and TLR4 [4] is closely related to the severity of asthma. The mechanism of action underlying pediatric massage in the prevention and treatment of pediatric asthma is described herein.

Materials and methods

General materials

One hundred sixty pediatric patients with chronic persistent asthma were enrolled, as follows: 35 children hospitalized in the Affiliated Hospital of Hebei Union University, Tangshan City, Hebei Province between January 2010 and December 2012; 46 children from the Fifth Affiliated Hospital of Guangzhou Medical University, Guangzhou City, Guangdong Province; 38 children from the Wuxi Third People's Hospital, Jiangsu Province; and 41 children from the Affiliated

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Hospital of Heilongjiang University of Chinese Medicine in Harbin, Heilongjiang Province. All of the enrolled patients were from the pediatric outpatient and inpatient departments. The 160 pediatric patients were equally divided into 2 groups (treatment and control groups) using a random allocation method. The inclusion criteria for the selected cases were as follows: Based on the *Convention of Pediatric Asthma Prevention and Control* formulated by the National Cooperative Group for Pediatric Asthma Prevention and Control, the severity of the asthma was Class 1 and 2 [5]; and the patients were 4–10 years of age. The exclusion criteria [6] were as follows: glucocorticoid therapy during the most recent 3 months; a history of a RTI in the most recent month; treatments with antihistamines, controlled-release theophylline, and anti-leukotrienes; severe primary diseases involving the heart, brain, liver, kidneys, and hemopoietic system; non-compliance with medications, thus rendering the curative effect immeasurable; loss to follow-up; and incomplete data, thus affecting interpretation of the curative effect. There was comparability, but no significant difference between the two groups with respect to age, gender, and disease course (P>0.05; Table 1). The authors received consent from all participants.

Therapeutic method

Control group: Beclomethasone aerosol (approval number: H37022928; Shandong JINGWEI Pharmacy, China) was administered for a 3-month observation period (200 μg bid).

Treatment group: The patients in the treatment group received pediatric massage to clear the lung and liver channels and observed for 3 months, as follows: tone the spleen, lung, and kidney channels for 15 min; run the Bagua inversely for 10 min; massage 3 strategic passes (exterior); joggle 6 hollow organs (interior) 100 times; rub Tiantu 50 times and Dingchuan 150 times; massage Shanzhong 20 times; roll the Rupang and Rugen 150 times; thread the Feishu 150 times; open Tianmen 20 times; joggle Kangong 20 times; and joggle Taiyang 20 times.

Table 1. General materials

Group	*n*	Gender		Age (years)	Disease course (months)
		Male	Female		
Treatment group	80	38	42	6.54±2.57	20.74±10.97
Control group	80	36	44	7.12±2.19	22.11±9.66

Detection method

The frequency of asthmatic attacks and RTIs of pediatric patients before treatment and during the 3-month treatment period were recorded based on questionnaire responses; the childhood asthma control test (C-ACT [Chinese version]) was adopted for scoring [7]. The Vmax pulmonary function instrument manufactured by American SensorMedics Company was used to conduct pulmonary function tests per American ATS standards; specifically, the peak expiratory flow (PEF) during the day and night was recorded every day, and the PEF% (measured PEF value/predicted PEF value×100%) was calculated.

Expression of macrophage TLR1, TLR2 and TLR4 [8]: Four mL of antecubital venous blood was collected from fasting pediatric patients before and the morning after treatment, and kept at –20°C. Whole blood samples were analyzed using flow cytometer via bi-color immunofluorescence direct labeling method. For each subject, 300 μL of anticoagulant whole blood was obtained and aliquoted in three test tubes (two for testing and one for control). Anti-fluorescein isothiocyanate monoclonal antibody (McAb; 10 mg/L) was added to each tube. Anti-TLR1-PE, anti-TLR2-PE, and anti-TLR4-PE (10 mg/L) were added to 2 tubes, and homotype control IgG2a (10 mg/L) was added to the control tube, and incubated for 30 min in a dark environment. The antibody was provided by Beijing Zhongsheng Ruikang Science and Technology Co., Ltd. (Beijing, China). Macrophages were obtained through CyTOF mass spectrum FCM (Beijing Dongsheng Innovative Bio-technology Co., Ltd., Beijing, China), and data were analyzed by FCSExpress 4.07 software. With respect to the expression of TLR1, TLR2, and TLR4, the homotype control was used to correct non-specific dying, and the results were expressed by the TLR relative average fluorescence intensity of cells, as follows: TLR relative average fluorescence intensity=TLR average fluorescence intensity/average fluorescence intensity of homotype control.

Criteria for therapeutic effects: The criteria for therapeutic effects were established in accordance with the *Convention of Pediatric Asthma Prevention and Control* [9], as formulated by

the National Pediatric Asthma Cooperative Group in 1998. Clinical control was defined as follows: the asthma symptoms were completely alleviated totally; in spite of slight attack, the asthma symptoms could be alleviated without drugs with a 1-s forced inspiratory volume (FEV1) or PEF increasing by >35%, or the (FEV1) or PEF≥80% of the predicted value after treatment, and the day-and-night variation rate of PEF<20%. Conspicuous effect was defined as follows: asthmatic attack clearly alleviated when compared with before treatment, and the FEV1 or PEF increased by 25%–35% or reached 60%–79% of the predicted value of FEV1 or PEF after treatment, with a day-and-night variation rate of PEF<20%, but the patient still needed glucocorticoids or bronchodilators. Improvement was defined as follows: the asthma symptoms were alleviated somewhat, and the FEV1 or PEF increased by 15%–24%, but the patient still needed glucocorticoids or bronchodilators. Ineffectiveness was defined as follows: clinical symptoms and the measured value of FEV1 or PEF were not improved or were exacerbated.

Statistical methods

SPSS 17.0 statistical software was adopted for analysis, with the data expressed by x±s. A t-test for independent samples was used to perform inter-group comparisons, and a $P<0.05$ indicated statistical significance.

Results

There were 80 cases in the treatment group, with 3 lost to follow-up during the therapeutic process, and 80 cases in the control group, with 4 lost to follow-up in the therapeutic process.

The frequency of asthmatic attacks and RTIs in the two groups post-treatment were significantly reduced ($P<0.05$) when compared with pre-treatment, and the C-ACT score and PEF% of the two groups after treatment were significantly increased ($P<0.05$). After treatment, the frequency of asthmatic attacks, the frequency of RTIs, the C-ACT score, and the PEF% in the treatment group differed significantly ($P<0.05$) from the control group. To summarize, the treatment group was better controlled than the control group (Table 2).

After treatment, the intensity of fluorescence representing the expression of TLR1, TLR2, and TLR4 of the two groups increased ($^{*}P<0.05$) substantially compared with the pre-treatment intensity. After treatment, the intensity of fluorescence representing the expression of TLR1, TLR2, and TLR4 of the treatment group compared with the control group was significantly different ($^{☆}P<0.05$). To summarize, the treatment group had increased expression of TLR1, TLR2, and TLR4 compared to the control group (Table 3).

The total effective clinical rate in the treatment and control groups was 90.79% and 77.92%, respectively ($P<0.05$) (Table 4).

Discussion

TLR is a pathogen pattern recognition receptor with innate immunity that mediates multiple panimmune cells, activates inflammatory factors, and regulates inflammatory reactions [10]. TLR1, TLR2, and TL4R are primarily expressed on multiple inflammatory cytomembranes and recognize cell wall elements and viral particles of bacteria. TLR1 recognizes microbial lipopeptides. TLR2 and TLR4 can recognize bacterial lipopolysaccharides (LPS) and lipoprotein. TLR2 binds with peptidoglycan of *Staphylococcus* to release histamine, and to secrete cell factors, such as IL-4, IL-6, IL-5, IL-13, and TNF-α, so as to mediate the inflammatory reaction with TLR2 cells as dominant, and to strengthen the TLR1 immune response to control chronic infections by *Mycobacterium*

Table 2. Comparison of the two groups of children with asthma in terms of frequency of asthmatic attacks, frequency of RTIs, C-ACT score, and PEF% (X±S)

Group	*n*	Frequency of asthmatic attacks		Frequency of RTIs		C-ACT score		PEF %	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group	77	2.61±0.64	2.12±0.47*	3.84±0.84	2.56±0.42*	14.67±1.65	17.34±1.87*	68.45±5.23	83.11±9.78*
Treatment group	76	2.58±0.71	1.57±0.54$^{*,☆}$	3.71±0.77	1.91±0.59$^{*,☆}$	15.05±1.34	21.56±2.13$^{*,☆}$	71.67±6.67	91.45±8.60$^{*,☆}$

Note: when compared to pre-treatment, $^{*}P<0.05$; when compared with the control group post-treatment, $^{☆}P<0.05$.

Table 3. Expression of PB macrophage TLR1, TLR2, and TLR4 in the two groups (X±S)

Group	*n*	TLR1		TLR2		TLR4	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group	50	1.23±0.19	1.43±0.18*	1.31±0.22	1.43±0.20*	1.59±0.25	1.68±0.22*
Treatment group	50	1.24±0.20	1.51±0.17*,☆	1.34±0.24	1.54±0.21*,☆	1.54±0.30	1.82±0.24*,☆

Note: Compared with pre-treatment, *P<0.05; compared with the control group post-treatment, ☆P<0.05.

Table 4. Comparison of the clinical effect in the two groups of pediatric patients

Group	Case number	Clinical control/case	Conspicuous effect/case	Effective/ case	Ineffective/ case	Effective rate/%
Control group	77	14	21	25	17	77.92
Treatment group	76	25	26	18	7	90.79☆

Note: When compared with the control group after treatment, ☆P<0.05.

tuberculosis. TLR4 binds with lipopolysaccharides of gram-negative bacteria to secrete cell factors, such as TNF, IL-6, and IL-13, and to induce the inflammatory reaction with TLR1 cells as dominant to effectively control the role of asthma in innate immune responses [11–16].

Pediatric massage [17–21] has achieved a prominent curative effect in the prevention and treatment of pediatric asthma, which has been widely acknowledged and applied by medical workers and parents of pediatric patients, and has become one of the common external therapies to prevent and treat pediatric asthma. Traditional pediatric massage manipulation was adopted in the current study to interfere with pediatric asthma patients in the chronic remission period so as to observe the frequency of asthmatic attacks, the frequency of RTIs, the C-ACT score, the PEF%, and the change in the expression of PB macrophage TLR1, TLR2, and TLR4. As a result, we showed that pediatric massage effectively enhanced the expression of PB macrophage TLR1, TLR2, and TLR4 in pediatric patients with asthma, activated numerous inflammatory factors, and strengthened the immune response to control chronic infections and improved the clinical effect. Pediatric asthma is subject to the influence of numerous inflammatory cells and relevant inflammatory factors. In ancillary studies, we will further explore the law of changes of TLR expression in children with pediatric asthma to further study the change in innate immune function and to seek new targets of pediatric massage to prevent and treat asthma.

Conflict of interest

The authors declare no conflict of interest.

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